IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: ANDERSON, Norman Leigh	)	Confirmation No: 6420
Application Serial No.: 10/676,005	)	Group Art Unit: 1655
Filed: October 02, 2003	)	Examiner: Jana A. Hines
	)	Attorney Docket: 15503-001US

For: ***HIGH SENSITIVITY QUANTITATION OF PEPTIDES BY MASS SPECTROSCOPY***

Supplemental Declaration under 37 C.F.R. § 1.132

I, Dr. Steven Carr, declare and say:

1. I am Director of Proteomics and Biomarker Discovery at the Broad Institute of Harvard and MIT, Cambridge Massachusetts.

2. On March 10, 2008, I submitted a declaration in connection with the captioned application. A copy of my *Curriculum Vitae* is appended as APPENDIX A to that declaration.

3. I have reviewed the Office Action issued December 8, 2008 in relevant part, and I have considered the Examiner's comments regarding my March 10, 2008 declaration. I have reviewed the specification of the captioned application, and I have reviewed the pending claims in Appendix B, below.

4. I respectfully disagree with the Examiner's assertion with regard to claim 60 that the specification does not show that the inventor possessed "the first and second peptides being selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer." A skilled scientist working in this field would understand that peptides that "ionize well" and "are detected at highest relative abundance" are those that provide a high signal to noise relative to peptides that do not ionize well or are detected at lower relative abundance. Accordingly, I conclude that the specification demonstrates that the inventor clearly possessed the subject matter of claim 60.

5. To the extent that the Examiner asserts that the declaration refers only to the system described in the application, and not to the individual claims, I respectfully submit that my comments apply to both the system described in the application and the claims.

6. I have considered the Geng and Little references cited by the Examiner, together with the Examiner's statement that "Dr. Carr states that there was no expectation that antibodies

or any other specific binding agent could be used to select peptides from a complex sample digest, however, contrary to Dr. Carr's statement; the art teaches selection of peptides using specific binding agents, from a digested complex serum sample." The Examiner's statement is based upon an erroneous reading of the references. My comments below focus on the use of antibodies as recited in the claims.

7. Contrary to the Examiner's assertion that "the art teaches selection of peptides using specific binding agents from a digested complex serum sample," neither the Geng nor Little references relied upon by the Examiner teaches the use of antibodies for such a selection. The Examiner admits that Geng does not teach the use of antibodies; accordingly, only the Little reference is available to teach the use of antibodies. However, Little fails to teach or suggest the use of antibodies to purify peptides from a proteolytic digest, whether or not that digest is a complex serum sample. Rather, Little describes a conventional use of antibodies for isolation of intact proteins **prior** to digestion by any protease. As such, Little describes isolation of proteins, not peptides, from mixtures that are drastically less complex than those produced by protease digestion. For at least these reasons, Little does not provide a sound scientific basis upon which to refute my earlier declaration.

8. The position taken by the Examiner assumes that antibodies can be raised against any peptide at will, and that such antibodies will be effective in isolating the peptide from a complex digest, such as a digest resulting from proteolysis of a biological fluid. The Examiner's assumption is incorrect, and fails to reflect the understanding in the field in 2002. Not all peptides are immunogenic, and the fact the antibodies can be raised against a protein containing a particular amino acid sequence neither guarantees that an antibody against a much shorter peptide will be present in the immune response nor that any antibody will be suitable for capturing the desired peptide.

9. Moreover, a skilled scientist in 2002 would not have had a reasonable expectation of success in using antibodies to isolate peptides from a proteolytic digest of a bodily fluid sample. Such proteolytic digests are complex and contain the desired peptides (often at very low concentrations) in the presence of a huge number of extraneous peptides. That being the case, any antibody has to be not only specific for the desired peptide, it must also have a suitably high affinity for that peptide so that it can specifically bind the peptide in the presence of huge numbers of competing peptides present at much higher concentrations. Furthermore, a skilled artisan would also recognize that to be very effective in the claimed method, antibodies would

have to specifically bind peptides that are readily ionized in a mass spectrometer. For at least these reasons, at the time that the captioned application was filed there was no reasonable expectation that an antibody could be successfully used to select one peptide from a complex digest resulting from a biological sample where that peptide would be a suitable peptide for purposes of the method described in the claims of the application.

9. It was therefore surprising to me and to other workers in the field when it was shown that the methods described and claimed in the captioned application were able to identify target peptides from complex digests of bodily fluids, and that the methods could be used to study proteins that are present at low concentration (low ng/ml) in such fluids.

10. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date: 7 June, 2009

Dr. Steven Carr

Appendix B

1-43. (Canceled)

44. (Previously Presented) A method of quantifying an amount of at least a first monitor peptide and a second monitor peptide in a biological sample, comprising:
contacting the sample with
(i) a first anti-peptide antibody specific for said first peptide and;
(ii) a known quantity of a labeled version of said first peptide;
contacting the sample with
(I) a second antipeptide antibody specific for said second peptide, wherein said second antibody is different from said first antibody and;
(ii) a known quantity of a labeled version of said second peptide, separating peptides bound by said first and said second antibodies from unbound peptides;
eluting said peptides bound by said first and said second antibodies from said antibodies;
measuring the amount of said first peptide eluted from said first antibody using a mass spectrometer;
measuring the amount of said labeled version of said first peptide eluted from said first antibody using a mass spectrometer;
calculating the amount of the first peptide in the biological sample;
measuring the amount of said second peptide eluted from said second antibody using a mass spectrometer;
measuring the amount of the labeled version of the second peptide eluted from said second antibody using a mass spectrometer; and
calculating the amount of the second peptide in the biological sample, wherein said biological sample is a proteolytic digest of a bodily fluid sample.

45-47. (Canceled)

48. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a monoclonal antibody.

49. (Previously Presented) The method of claim 44, wherein at least one of said first and said second antibodies is a polyclonal antibody.

50. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both polyclonal antibodies.

51. (Previously Presented) The method of claim 44, wherein said first and said second antibodies are both monoclonal antibodies.

52-53. (Canceled)

54. (Previously Presented) The method of claim 44, wherein the labeled version of the first peptide includes at least one site at which a stable isotope is substituted for the corresponding predominant natural isotope in more than 98% of peptide molecules.

55. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a support.

56. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a packed column.

57. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to a monolithic porous support.

58. (The method of claim 44, further comprising: attaching the first antibody to a mesh.

59. (Previously Presented) The method of claim 44, further comprising: attaching the first antibody to magnetic beads.

60. (Previously Presented) The method of claim 44, wherein the first peptide and the second peptide are selected from among the set of peptides produced by digestion of the target protein to provide high signal to noise in the mass spectrometer.

61. (Previously Presented) A method for quantifying the amount of a peptide, comprising:
contacting the sample with
(i) an anti-peptide antibody specific for said peptide;
(ii) a known quantity of a labeled version of the peptide,
separating peptides bound by said antibody from unbound peptides
eluting said peptide bound by said antibody from said antibody;
measuring the amount of the peptide eluted from said
antibody using a mass spectrometer: and
calculating the amount of the peptide in the biological sample;
wherein said biological sample is a proteolytic digest of a bodily fluid.

62-63. (Canceled)

64. (Previously Presented) The method of claim 61, further comprising: preparing the labeled version of the peptide.

65. (Previously Presented) The method of claim 61, wherein the labeled version of the peptide includes at least one site at which a stable isotope is substituted for the predominant natural isotope in more than 98% of peptide molecules.

66-70. (Canceled)

71. (Currently Amended) The method of claim 44, further comprising:
preparing the labeled version of the monitor peptide.

72. (Currently Amended) The method of claim 71, wherein the labeled version of the monitor peptide includes a stable isotope.

73. (Canceled).

74. (Previously Presented) method of claim 44, wherein said first anti-peptide antibody is created using said first peptide or a nonmaterially modified version of the first monitor peptide.

75. (Previously Presented)) The method of claim 44, further comprising: creating the first antibody using the first peptide or a non-materially modified version of the first peptide.

76. (Canceled).

77. (Previously Presented) The method of claim 61, further comprising: creating the anti-peptide antibody using the peptide or a non-materially modified version of the peptide.

78. (Currently Amended) The method of claim 44, wherein the said bound peptides are subjected to a chromatography step after elution from said antibodies and before introduction into said mass spectrometer.

79-80. (Canceled)

81. (Currently Amended) The method of claim 61, wherein said bound peptides are subjected to a chromatography step after elution from said antibody and before introduction into said mass spectrometer.

82. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a polyclonal antibody.

83. (Previously Presented) The method of claim 61, wherein the anti-peptide antibody is a monoclonal antibody.

84. (Previously Presented) The method of claim 44 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

85. (Previously Presented) The method of claim 61 wherein said first and second peptides are proteolytically cleaved from first and second sample proteins, respectively, and wherein the amounts of said first and second proteins in said body fluid sample are calculated from the amounts of said first and said second peptides in the sample.

86. (Previously Presented) The method of claim 61, wherein the polyclonal antibody is created using the monitor peptide or a non-materially modified version of the monitor peptide.